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FOR MORE INFORMATION
Leemor Joshua-Tor
W.M. Keck Structural Biology Laboratory
Cold Spring Harbor Lab
leemor@cshl.edu

STRUCTURAL BASIS FOR THE EBA-175 ERYTHROCYTE INVASION PATHWAY OF THE MALARIA PARASITE *PLASMODIUM FALCIPARUM*

N.H. Tolia[1,2], E.J. Enemark[2], B. Kim Lee Sim[3], and L. Joshua-Tor[1,2]

[1]Watson School of Biological Sciences and [2]Keck Structural Biology Laboratory, Cold Spring Harbor Laboratory; [3]Protein Potential

Invasion of red blood cells by the malaria parasite Plasmodium falciparum marks the commencement of the clinical manifestation of the disease. During invasion, erythrocyte binding antigen 175 (EBA-175), a protein on the surface of the parasite, binds to glycophorin A (GpA) on red blood cells. Of the several domains in EBA-175, region II (RII) is necessary and sufficient for binding to GpA. We have solved the crystal structures of RII and RII in complex with a sugar that contains the essential components required for the binding of EBA-175 to GpA. This study provides insight into the mechanism of erythrocyte binding and invasion by the malaria parasite, and understanding this interaction will aid in the development of drugs and vaccines.

Malaria causes an estimated 300-500 million cases and 1-3 million deaths annually, 80% of which are in children under the age of five. There are four species of malaria parasite, of which *Plasmodium falciparum* is responsible for the majority of deaths associated with the disease. Within 9-14 days after an organism is infected (via a mosquito bite) the parasite has invaded the red blood cells, breaking them down and resulting in the clinical symptoms of the disease. Therefore, preventing binding and invasion of the red blood cells by the parasite would be critical in either preventing malaria (through vaccine design) or treating it (through drug design).

Binding of the parasite to the red blood cells requires the interaction between erythrocyte binding antigen 175 (EBA-175), a protein on the surface of the parasite, and the sugars of glycophorin A (GpA) on the red blood cell. One domain of EBA-175, region II, has been found to be the essential component of the parasite required to bind GpA. The structure of RII shows that it is a dimer, and that the two molecules interact extensively with one another in an anti-parallel fashion resembling a handshake. The dimer results in the formation of two channels in the center of the molecule (**Figure 1**).

Authors (from left) Eric Enemark, Leemor Joshua-Tor, and Niraj Tolia

To examine the binding interaction of RII to GpA, we cocrystallized RII with the sugar α-2,3-sialyllactose, which contains the essential components of GpA required for binding to RII. We found six binding sites for the sugar to the RII dimer, all of which were located at the dimer interface (**Figure 1**). Using a cell-based binding assay, we mutated RII at residues required for dimerization or the observed sugar binding sites to confirm that dimerization and sugar/RII binding are essential for red blood cell binding. These mutations were expressed on the surface of COS cells (monkey test cells) and we assayed for their ability to bind normal human erythrocytes. All dimerization and glycan-binding mutants had impaired erythrocyte-binding ability, demonstrating the importance of dimerization in erythrocyte binding and validating the glycan binding sites.

Together these results allowed us to propose a model for RII binding to GpA (**Figure 2**). We propose that the RII domain of EBA-175, which is present on the surface of the parasite, might assemble around the GpA extracellular domains of the red blood cells and that dimerization of EBA-175 would occur upon receptor binding. The GpA extracellular domains may either bind within the channels or dock on the outer surface of RII, feeding sugars into the channels. Binding of the

malaria parasite to the red blood cell triggers many signaling pathways within the red blood cell. Therefore, we propose that RII-mediated dimerization of EBA-175 may be the trigger for signaling during invasion.

These structures have broad implications in helping to model other erythrocyte-binding-like (EBL) family members required for binding between the parasite and red blood cells. Of particular interest is the homologous protein PfEMP-1 that is responsible for cytoadherence of the parasitized red blood cells to the endothelium, resulting in the most fatal form of malaria. Perhaps most importantly, the structure, along with the functional analysis, presents possibilities for drug and vaccine design.

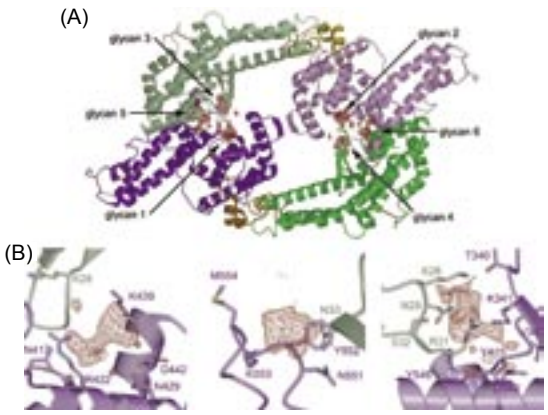

Figure 1. Crystal structure of RII with sialyllactose. (A) A ribbon representation of the dimeric structure of RII. The monomers are shaded in different intensities and are composed of two subdomains (green and purple), F1 and F2. Two channels are created in the center of the molecule. Glycan positions are shown in $F_o - F_c$ electron density in red. (B) Close-up views of three of the glycan binding sites – 1, 3 and 5, from left to right. Residues from both monomers contact each glycan.

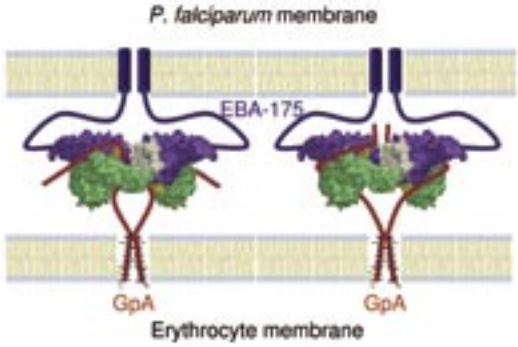

Figure 2. The P. *falciparum* membrane is shown on the top and the erythrocyte membrane on the bottom. The receptor binding domain of EBA-175, RII, is shown as a surface representation (green/purple denotes each sub-domain). Blue lines represent portions of EBA-175 backbone not included in the crystal structure. The receptor Glycophorin A (GpA) is shown in red with the membrane-spanning region in detail using the NMR structure and the extracellular domain is drawn as a schematic flexible line. The glycans of GpA, modeled based on the position of sialyllactose, are shown as space-filling models in gold. In the left panel, the RII dimer assembles around the GpA dimer, with GpA binding within the channels. An alternative model is shown on the right, where the GpA monomers dock on the outer surface of the protein, feeding glycans into the channels.